REMARKS/ARGUMENTS

Claims 1-69 have been subjected to substantive examination. No claims have been amended by this response. Applicants respectfully request the Examiner reconsider the pending claims in light of the remarks below.

Provisional Nonstatutory Obviousness-type Double Patenting:

Claims 40 and 60-69 stand provisionally rejected on the ground of nonstatutory obviousness-type double patenting, the Examiner alleging that the claims are unpatentable over claims 1-26 of copending Application No. 10/992,154. The Examiner has further alleged that although the conflicting claims are not identical, they are not patentably distinct from each other because they commonly comprise methods of using cross-flow membrane filtration to enrich recirculating solutions in leukocytes so as to culture stem cell populations. This has been stated as a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Applicants have noted, the above provisional rejection of claims 40 and 60-69 and will take whatever action might be appropriate when patentable subject matter has been recognized.

Rejections Under 35 U.S.C. § 102:

Claims 25-32 stand rejected under 35 U.S.C. 102(b) as being anticipated by Castino patent 4,420,398. The Examiner has alleged that Castino *et al.* discloses a method and system for separating leukocytes from blood sources originally obtained as whole blood samples from human patients or donors. The Examiner further alleges that Castino *et al.* disclose that blood is introduced into cross-flow membrane-containing remover units 11 and 21 through respective inlets where leukocytes are selectively removed from other blood components and constituents to form cell populations that are enriched for leukocytes. In addition, the Examiner alleges that Castino *et al.* teach that the respective retentate populations are continuously

recirculated to cell populations enriched in forms of leukocytes ("production broth" and CPAS reservoir, respectively. Still further, for claims 26-32, the Examiner alleges that the cell populations of Castino *et al.* are prepared by upstream filtration or leukapheresis, and that the blood constituents naturally contain plasma, platelets, erythrocytes, *etc.*, and that the recycling of stream volumes may be carried out indefinitely (column 14, lines 45-50).

Applicants respectfully disagree with the rejection of claims 25 through 32 as anticipated by Castino *et al.* In particular, the Examiner alleges that Castino *et al.* disclose a method and system for separating leukocytes from blood sources originally obtained as whole blood samples from human patients or donors and that Castino *et al.* disclose the introduction of blood into a cross-flow chamber for separation of leukocytes. Applicants respectfully direct the Examiner to column 4, line 63 through column 5, line 4, wherein Castino *et al.* describe their invention as a method for extracting cell produced anti-viral substances from a production broth comprising subjecting the broth to a cross-flow membrane filtration wherein the broth in passed over a membrane filter and the cell produced anti-viral substance (CPAS) passes through the membrane. Further, Applicants direct the Examiner to column 6, lines 3-13 wherein Castino *et al.* describe the cross-flow filtration technique of their invention as a "high pass/low pass" process in that a first filtration separates the CPAS and smaller particles from larger particles (the cells that produced the CPAS). Where a recirculation is used, the production cells are returned to the production broth intact. The described process and device does not disclose an apparatus or system that enriches a blood sample for leukocytes. Further, there is no suggestion or disclosure of a fluid outlet centrally disposed in a portion of the cross-flow chamber opposite the filter surface. Castino *et al.* also do not disclose the introduction of a blood sample into the device, but instead introduce a production broth that can comprise a cell culture medium with, for example, leukocytes. The method and device is intended to separate proteins produced by the cultured cells from the cells and other cell culture products, not to separate leukocytes from other blood constituents. In addition, the device of Castino *et al.* also does not disclose or suggest use of a filter having a pore size of about 1 to about 10 microns. In fact, at column 6, lines 49-59, Castino *et al.* disclose the use of a first filter having a pore size, or molecular weight cut off,

"sized to pass all molecules and particles having a molecular weight smaller than at least 40,000 daltons, and preferably up to 100,000 daltons." A filter having a pore size in the disclosed range would not be useful in the methods of the present invention as red blood cells and certain white blood cells would not be capable of passing through the membrane.

In addition, for claims 26-32, the Examiner alleges that the cell populations of Castino *et al.* are prepared by upstream filtration or leukapheresis, and that the blood constituents naturally contain plasma, platelets, erythrocytes, *etc.*, and that the recycling of stream volumes may be carried out indefinitely (column 14, lines 45-50). Castino *et al.* do obtain the leukocytes used in their methods from processes that include plateletpheresis, but the remaining process was designed to obtain white blood cell populations for *in vitro* cell culture. The steps included red blood cell lysis and centrifugation. The cell population was cultured and induced for the production of the CPAS and the filtration process was used to separate the CPAS from the cell culture. Recycling of the filtrate could be carried out indefinitely, but there is no enrichment of leukocytes as none of the cells in the cell culture are intended to pass through the filter. As such, there in nothing in the teachings of Castino *et al.* that disclose or suggest the device or the methods of claims 26-32.

Applicants respectfully request the Examiner to reconsider and withdraw the rejection of claims 25 through 32 under 35 U.S.C. 102(b) as being anticipated by Castino patent 4,420,398.

Rejections Under 35 U.S.C. § 103:

Claims 1-10, 12, 14, 33, 40-48 and 60 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Castino (*supra*) in view of Gsell *et al.* (US patent 5,695,563). As above, the Examiner alleges that Castino *et al.* discloses a method and system for separating leukocytes from blood sources originally obtained as whole blood samples from human patients or donors. Further, the Examiner alleges that Castino *et al.* teach that the blood is introduced into cross-flow membrane-containing remover units 11 and 21 through respective inlets where leukocytes are selectively removed from other blood components and constitutents to form cell populations that are enriched for leukocytes. The Examiner further alleges that the respective retentate

populations of Castino *et al.* are continuously recirculated to cell populations enriched in forms of leukocytes ("production broth" and CPAS reservoir, respectively). In addition, for the dependent claims, the Examiner alleges that Castino also discloses the following: i) the cell populations being prepared by samples upstream filtration or leukapheresis; ii) the blood constituents naturally contain plasma, platelets, erythrocytes, etc., and iii) the recycling of stream volumes may be carried out indefinitely (column 14, lines 45-50), (claims 42-47). Castino is further alleged by the Examiner to disclose means for heating to control temperature and means to control of filtration flow rates (claim 21), filter pore size of about 3-5 microns or adapted to retain leukocytes (claims 3, 4, 41, 46, 47), blood sources (claims 5, 6), recovery unit and crossflow filters being in loop format and connected by inlets and outlets to the units (for claims 7-10, 49 and 50), means for culturing (claims 14). These claims all differ in requiring that the inlet be disposed to introduce the blood parallel to, or tangential to the surface of the filter and the outlet being centrally disposed, and/or by requiring a vortex motion. The Examiner believes that Gsell teaches or infers such arrangement of inlets and outlets citing the illustrations in figures 1-7 and by the discussion of spiral flow or vortex motion. From these alleged disclosures, the Examiner has asserted that it would have been obvious to one of ordinary skill in the art to have constructed the filtration cells of Castino *et al.* to have the tangential inlet, vortex/spiral flow and central outlets of Gsell, so as to minimize stresses to the various types of cells in the blood being separated, and increase separation efficiency by causing flow of the blood over a larger surface area of filter surface during processing.

Applicants strongly disagree with this rejection and respectfully request the Examiner reconsider the cited references and the alleged teachings of the references. Castino *et al.* is described above. As to this particular rejection, Castino *et al.* does not disclose or suggest the separation of blood components by cross-flow filtration. Instead, Castino *et al.* teach the separation of cell produced antiviral substances, typically proteins, from the *in vitro* cultured cells that produced the substances. There is no suggestion or disclosure that tangential flow filtration methods of the present invention could be used to separate different cell types found in a blood product such as a plasmaphoresis or leukapheresis product. Although Castino *et al.* does

teach certain dependent elements of the present invention, such as blood sources, use of a recovery unit and a temperature control device, none of these elements are disclosed in a context that would suggest the device or methods of the present invention. In particular, although Castino *et al.* disclose isolating leukocytes from a plasmaphoresis device, the product is enriched for leukocytes by lysing red blood cells and removing other contaminants by centrifugation and other methods. The isolated leukocytes are cultured *in vitro* to produce the cell produced antiviral substance (CPAS) and the cross-flow filtration device and methods of Castino *et al.* are used to separate the CPAS from the leukocytes and not to enrich the leukocytes from the other cellular constituents of a blood product. Further, the temperature control means is provided in Castino *et al.* to cool the protein product in the filtrate and to warm the cells and other large particles in the retentate as it recirculates back to the production broth. This disclosure does not suggest the use of tangential flow filtration to separate cells of a blood product or the combination of elements in the device claimed in the instant application when considered alone or in combination with any of the other cited references.

Gsell *et al.* is cited as disclosing a centrifugal motion that the Examiner alleges can be combined with the method of Castino *et al.* for purifying the production broth. This combination is asserted by the Examiner to suggest the present invention as claimed. Gsell *et al.* disclose a biological fluid processing system for the removal of platelets from a blood product. Certain embodiments of the invention describe an embodiment that includes a spiral flow path, but the inlet (11) is not disposed to introduce a sample parallel to the surface of the filter. Further, the system includes a fibrous filter for the removal of leukocytes from the plasma depleted or from the plasma rich fluid that passes through the system. The spiral flow described in Gsell *et al.* is the result of flow channels in the surface of the separation medium. The device of the present invention does not involve the use of spiral flow channels in the surface of the filter. Any combination of Castino *et al.* and Gsell *et al.* does not disclose or suggest the device or methods of the present invention.

Claim 11 stands rejected under 35 U.S.C. 103(a) as being unpatentable over Castino *et al.* (*supra*) in view of Gsell *et al.* (US 5,695,563) as applied to claims 1-10 above, and

further in view of Raehse *et al.* (US 4,751,003) or Harm *et al.* (US 4,722,902). The Examiner has noted that Claim 11 additionally requires the crossflow chamber to be cylindrical and alleges that both Raehse and Harm show such cylindrical filter membranes for crossflow separation of blood components. The Examiner further alleges that it would have been also obvious to have utilized cylindrical filter membranes of Raehse or Harm to enhance separation efficiency, since crossflow in such blood separations has been shown to result in near 100% separation rates citing the Abstract of Raehse.

Applicants again strongly disagree with the rejection of claim 11 as being unpatentable over Castino *et al.* in view of Gsell *et al.* and further in view of Raehse and Harm. Castino *et al.* and Gsell *et al.* as they have been applied to claims 1 through 10 are described above. Claim 11 is directed to the device of claim 1 or claim 2 wherein the cross-flow chamber is cylindrical and the outlet is located opposite the center of the filter and perpendicular to a surface of the filter. Raehse *et al.* disclose a process for the separation of biotechnologically produced valuable materials from a cell culture suspension by cross-flow microfiltration using a porous polymeric tube with micropores greater than 1 μm. Applicants can find no suggestion or disclosure of the elements of dependent claim 11 in Raehse *et al.* The tube of Raehse *et al.* could be considered a cross-flow chamber and filter, but the pores are dispersed throughout the surface of the tube, the outlet would then be considered the end of the tube in the direction of flow. With this configuration it does not appear to be possible that the outlet could be oriented such that it is opposite the center of the filter. Harm *et al.* discloses of cell culture system wherein the culture medium is passed through a loop comprising a cylindrical fiber filter to remove waste material from the culture medium. There is nothing in Harm *et al.* that completes the disclosures of Castino *et al.*, Gsell *et al.* and/or Raehse *et al.* that discloses or suggests the present invention as recited in claim 11. None of the cited references when considered alone or in any combination disclose or suggest the separation of different cell types found in a blood product. Further, there is nothing in any of the references when considered alone or in any combination that discloses or suggests the combination of elements that comprise the device as recited in claim 11. Applicants respectfully request the Examiner to reconsider and withdraw the rejection of claim 11.

Claims 13, 15-22, 34-39, 49-59 and 61-69 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Castino *et al.* (*supra*) in view of Gsell *et al.* (US 5,695,563) as applied to claims 1-10, 12, 14, 33, 40-48 and 60 above, and further in view of Kopf (US 6,214,221) and/or Yamanishi *et al.* (US 2003/0134416 or US 6,949,355, based on provisional applications 60/394,517; 60/348,228 and 60/328,724, filed on 7/9/2002; 10/29/2001 and 10/11/2001, respectively). The Examiner notes that these claims differ in additionally requiring various means and method steps to enhance culturing of and growth of the concentrated leukocytes or substances being derived therefrom, including beads, cell-adhering substrate and screen, tissue culture vessel to receive mature cell cultures, separate mature from immature cell cultures, temperature control means, and wash and drain lines.

Yamanishi is alleged to teach cell culture media substrates and cell culturing and maturing method steps and system components (paragraphs 48, 71, 94, 96, 131-139 and 148 of US 2003/0134416). The Examiner further alleges that the patent (US 6,949,355) teaches the use of beads (column 7, lines 50- 67), washing and draining means (column 5, lines 53-column 6, line 12), antigen/antibody binding substances and substrates (column 8, lines 5-38), culture of stem cells (column 9, lines 57-67), separation of mature from immature cells (column 11, lines 20-49). Yamanishi is also alleged to teach such cell culture media substrates and cell culturing and maturing method steps and system components (paragraphs 48, 71, 94, 96, 131-139 and 148 of US 2003/0134416).

Kopf is alleged by the Examiner to teach cell culture media substrates and cell culturing and maturing method steps and system components (column 7, lines 50-67), washing and draining means (column 10, lines 47-50), antigen/antibody binding substances and substrates (column 9, lines 35-53), culture growth, nurturing \and derivation of populations of cells (column 14, lines 35-40), separation of mature from immature cells (column 11, lines 20-49). The patent teaches use of beads (column 12, lines 25-35), separation of mature from immature cells (column 11, lines 20-49),and temperature control (column 13, lines 37-47).

The Examiner believes that it would have been obvious to have augmented the crossflow filtration loop and recycling system and method of Castino, with the various means to

culture leukocyte-derived substances and cells and stem cells, as suggested by Yamanishi or Kopf, so as to both enrich leukocyte-product cell populations and promote growth and maturing of cell cultures, so as to have a complete cell growth and culturing system in one convenient and central location, to avoid loss of cell populations and leukocyte ingredients that would otherwise result were transport of cell cultures between processing facilities.

As above, Castino *et al.* either alone or in combination with Gsell *et al.* do not disclose or suggest the invention as recited in the claims. The Examiner has cited Yamanishi *et al.* and/or Kopf *et al.* as disclosing or suggesting certain elements of dependent claims 13, 15-22, 34-39, 49-59 and 61-69. The dependent claims include all of the elements of the claims from which they depend. As claims 13, 15-22, 34-39, 49-59 and 61-69 depend ultimately from claim 1, Yamanishi *et al.* and/or Kopf *et al.* do not overcome the deficiencies in Castino *et al.* and/or Gsell *et al.* in disclosing or suggesting the present invention. Yamanishi *et al.* define certain terms including, for example, stem cell, microparticle, a means for culturing the cell population enriched for leukocytes, and the like. But, Yamanishi *et al.* do not disclose or suggest the tangential flow filtration device as set forth in claim 1. Similarly, Kopf *et al.* disclose a method and apparatus for purifying a target substance. The method and apparatus can comprise sequential chromatographic and diafiltration steps in a cross-flow filtration system. The cross-flow system is used in the methods to separate the target substance from any larger particles present in the system, including cells. These is no disclosure or suggestion of separating cells by a tangential flow filtration method or apparatus with elements as set forth in the present application.

Claims 23 and 24 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Castino *et al.* (*supra*) in view of Gsell *et al.* (US 5,695,563) and Krasnoff et al. (US 5,690,815). Castino *et al.* is alleged to disclose a method and system for separating leukocytes from blood sources originally obtained as whole blood samples from human patients or donors. The Examiner has summarized Castino *et al.* as teaching the introduction of blood into a cross-flow membrane-containing remover units 11 and 21 through respective inlets where leukocytes are selectively removed from other blood components and constituents to form cell populations

that are enriched for leukocytes. Further, the Examiner asserts that Castino *et al.* teach that respective retentate populations are continuously recirculated to cell populations enriched in forms of leukocytes ("production broth" and CPAS reservoir, respectively. For the dependent claims, the Examiner has alleged that Castino *et al.* also disclose the following: the cell populations being prepared by samples upstream filtration or leukophoresis , the blood constituents naturally contain plasma, platelets, erythrocytes, etc. and the recycling of stream volumes may be carried out indefinitely. Castino *et al.* is further alleged by the Examiner to disclose means for heating to controlled temperature and control of filtration flow rates, a filter pore size of about 3 - 5 microns or adapted to retain leukocytes, blood sources, a recovery unit and crossflow filters being in loop format and connected by inlets and outlets to the units, and a means for culturing.

As above, the Examiner has determined that these claims all differ in requiring that the inlet be disposed to introduce the blood parallel to, or tangential to the surface of the filter and the outlet being centrally disposed, and/or by requiring a vortex motion.

Gsell is alleged by the Examiner to teach or infer such an arrangement of inlets and outlets by illustrations in figures 1-7 and by discussion of spiral flow or vortex motion. Therefore, the Examiner believes that it would have been obvious to one of ordinary skill in the art to have constructed the filtration cells of Castino *et al.* to have the tangential inlet, vortex/spiral flow and central outlets of Gsell, so as to minimize stresses to the various types of cells in the blood being separated, and increase separation efficiency by causing flow of the blood over a larger surface area of filter surface during processing.

Claims 23 and 24 additionally are summarized by the Examiner as differing in requiring that the inlet and retentate be disposed in an upper chamber above the filter surface. Krasnoff teaches such orientation in column 9, lines 1-40. It would have been an obvious expedient to have oriented the system of Castino/Gsell vertically as shown in Krasnoff to facilitate system setup and in working rooms of laboratories and industrial settings.

As above, Castino *et al.* and Gsell *et al.* do not disclose or suggest a method for enriching a cell population comprising blood components in leukocytes or apparatus comprising

the elements set forth in the pending claims. In particular, the device of Castino *et al.* disclose a method for isolating a cell produced antiviral substance, typically a protein, from a cell culture system. The cells in the cell culture system can comprise leukocytes, but the leukocytes have been separated from other blood components by other standard methods including red cell lysis and differential centrifugation. Castino *et al.* also do not disclose or suggest the elements of the tangential flow filtration device of claims 23 and/or 24. Gsell *et al.*, as above, do not disclose or suggest those elements not present in Castino *et al.* to result in the invention as recited in claims 23 and 24. The Examiner has cited Gsell *et al.* in particular for disclosing a vortex, spiral flow. Applicants note that the vortex spiral flow of Gsell *et al.* is accomplished through the use of a spiral flow path etched into the filter surface not by placement of the sample inlet parallel to the surface of the filter. As such, Castino *et al.* and Gsell *et al.* when considered alone or in any combination do not disclose or suggest the invention as recited in claims 23 and 24.

Krasnoff *et al.* is alleged by the Examiner to teach the orientation of the elements of the tangential flow filtration device of claims 23 and 24. Krasnoff *et al.* disclose a blood processing system for removing leukocytes and/or red blood cells from a plasma rich or plasma depleted final blood product. The device can comprise a leukocyte depletion assembly (13) and/or a red cell depletion assembly (12). These assemblies can be combined in certain embodiments of the invention. No embodiment of the invention discloses a method or tangential flow filtration device with a retentate enriched for leukocytes that can be used to produce either dendritic cells or matured and differentiated hematopoietic stem cells. Combination of Krasnoff *et al.* with any of Castino *et al.* and/or Gsell *et al.* would not result in the invention as recited in claims 23 or 24. At most, one of skill in the art would add a leukocyte depletion assembly and/or a red blood cell depletion assembly prior to the filtration device of Castino *et al.* and/ or Gsell *et al.* This combination does not result in the invention as claimed.

Claims 61-69 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Castino *et al.* (*supra*) in view of Gsell *et al.* (*supra*) as applied to claims 1-10, 12, 14, 33, 40-48 and 60 above, and further in view of Yamanishi *et al.* (*supra*). The Examiner has asserted that "these claims differ in additionally requiring various means and method steps to enhance

culturing of and growth of the concentrated leukocytes or substances being derived therefrom, including beads, cell-adhering substrate and screen, tissue culture vessel to receive mature cell cultures, separate mature from immature cell cultures, temperature control means, and wash and drain lines." Further, Yamanishi *et al.* is alleged by the Examiner to teach such cell culture media substrates and cell culturing and maturing method steps and system components citing paragraphs 48, 71, 94, 96, 131-139 and 148 of the US 2003/0134416). The Examiner further alleges that the patent (US 6,949,355) teaches the use of beads, washing and draining means, antigen/antibody binding substances and substrates, culture of stem cells, separation of mature from immature cells. Based on these alleged teaching the Examiner believes that it would have also been obvious to have augmented the crossflow filtration loop and recycling system and method of Castino *et al.*, with the various means to culture leukocyte-derived substances and cells and stem cells, as suggested by Yamanishi *et al.*, so as to both enrich leukocyte-product cell populations and promote growth and maturing of cell cultures, so as to have a complete cell growth and culturing system in one convenient and central location, to avoid loss of cell populations and leukocyte ingredients that would otherwise result were transport of cell cultures between processing facilities.

As above, Applicants must disagree with the rejection of claims 61 through 69 as being unpatentable over Castino *et al.* in view of Yamanishi *et al.* Castino *et al.* do not disclose a method for the enrichment of leukocytes from a blood product by tangential flow filtration, nor is a method for the enrichment of $CD34^+$ stem cells by tangential flow filtration disclosed or suggested. Castino *et al.* disclose a filtration device for separating a cell produced antiviral substance from leukocytes that have been isolated by other methods. Yamanishi *et al.* do not add anything to the teachings of Castino *et al.* to disclose or suggest the methods of the present invention. Yamanishi *et al.* disclose a method for the isolation of hematopoietic stem cells from a blood product, but that method requires the use of a specific binding agent, such as a CD71 specific antibody for enriching for nucleated red blood cells. There is nothing in Yamanishi *et al.* to disclose or suggest that tangential flow filtration can be used to enrich $CD34^+$ stem cells

from red blood cells and other white blood cells by tangential flow filtration methods, or by using a device as claimed in the present application.

Applicants respectfully request the Examiner to reconsider and withdraw the rejections of claims 1 through 69 as being unpatentable under 35 U.S.C. 103(a) over Castino (*supra*) in view of Gsell *et al.* (US patent 5,695,563), and further in view of various combinations of Raehse *et al.* (US patent 4,751,003), Harm *et al.* (US patent 4,722,902), Kopf *et al.* (US patent 6,214,221), Yamanishi *et al.* (US 2003/0134416 and US patent 6,949,355), and Krasnoff *et al.* (US patent 5,690,815) in view of the above remarks.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested. If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 206-467-9600.

Respectfully submitted,

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